RAPID DIAGNOSTICS

Excellence in sexually transmitted infection (STI) diagnostics: recognition of past successes and strategies for the future

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Diagnostic advances do not generally receive the recognition given to prevention and treatment contributions, for the control and management of infectious diseases including sexually transmitted infections (STIs). In order to identify seminal diagnostic contributions over a half century (1950–2000), the Editorial Board of the WHO Sexually Transmitted Diseases Diagnostics Initiative (SDI) Publication Review or ''electronic journal club'' were asked to nominate their choices of peer-reviewed publications for special recognition. From 43 nominations, 13 were voted by a panel of 25 ''experts'' as having made the most significant contributions. The 1964 article by Thayer and Martin, which identified a selective media for gonococcal culture, was chosen unanimously by all panel members and is identified as the classic STI diagnostic article for this era.

Effective management for infectious illness rests on the three pillars of diagnosis, prevention, and treatment. The aetiologic diagnosis of infections, including sexually transmitted infections (STIs), is the basis of meaningful surveillance, effective public health interventions, and optimal disease management. For most of the past 50 years, the diagnostic pillar has been the least well supported. However, substantial laboratory infrastructure has been established throughout the developed world to ensure that epidemic disease and serious infections are diagnosed through an array of cultural, serologic, and molecular technologies. In particular, microbial aetiologic diagnoses have enabled epidemics to be identified early and contained or eradicated. It has also identified the gaps in our knowledge of infectious disease syndromes where microbial aetiology remains uncertain.

These diagnostic advances have occurred for pathogens known to be transmitted sexually. Most STIs are universally managed syndromically, but when resources are available, significant efforts are made to determine their aetiology. This enables partner notification and surveillance to occur and disease control programmes to be pathogen-specific. In resource-poor societies, the World Health Organization (WHO) recommends a syndromic approach to STI management because access to laboratory services is limited. Clinically ''determined'' microbial aetiologies in patients with STI syndromes are often incorrect.[1 2] Efforts to alter this with less expensive simple point-of-care (POC) tests are underway but currently, with the exception of rapid HIV tests, none is in common use in most resource limited societies. Most POC tests for STIs are neither sensitive nor specific.

The STD Diagnostics Initiative (SDI), currently housed in the UNICEF/UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (WHO/TDR), has prioritised the development, evaluation and application of STI diagnostics appropriate for resource-limited settings. With funding from the United States Agency for International Development and the Bill & Melinda Gates Foundation, SDI has set up a network of laboratory and field sites for rapid test evaluation and is working with industry and country programmes on the development and implementation of new STI diagnostics in the developing world.

SDI has set up a website (www.who.int/std_diagnostics) for the timely dissemination of information on STI diagnostics. Within the website, there is a publication review section where the latest findings in STI diagnostics in peer-reviewed journals are summarised and commented upon by experts in the field, the Editorial Board members. Currently over 80 experts from 15 countries are members of this board. This article attempts to ''jazz up'' this process by identifying and providing evidence for excellence among peer-reviewed articles that have substantially contributed to the field of STI diagnostics in the last 50 years and have made a difference in care or control.

In a recent Perspective in the *New England Journal of Medicine*, Markel highlighted some of the historical controversies with regard to ''Who's On First? - Medical Discoveries and Scientific Priority''; Koch versus Pasteur; Watson versus Franklin; Gallo versus Montagnier.[3] However, this effort to identify key contributions to STI diagnostics is not intended to create controversy. To quote Sir Isaac Newton, ''If I have seen further, it is by standing on the shoulders of giants''.[3] Markel concludes, ''The question of who was first is more of a parlor game than an issue of real historical or scientific concern, primarily because contemporaries all have access to the same 'giants' shoulders''.[3]

Selecting the more important articles from the substantial literature on STIs is daunting and perhaps even unwise. However, identifying excellence, celebrating achievements, and providing kudos for advances in science is worthwhile. Our selections may be idiosyncratic, but we have attempted to recognise among the hundreds of articles over half a century, those that contribute to STI diagnosis, including some that have particularly altered paradigms and have become STI icons.

Abbreviations: EIA, enzyme immunoassays; ELISA, enzyme-linked immunosorbent assay; FTA, fluorescent treponemal antibody; FTA-ABS, fluorescent treponemal antibody absorption; HPV, human papillomavirus; HSV, herpes simplex virus; MHA-TP, microhaemagglutination assay for *T pallidum* antibodies; MIF, microimmunofluorescence; M-PCR, multiplex polymerase chain reaction; NAA, nucleic acid amplification; NAH, nucleic acid hybridisation; PCR, polymerase chain reaction; POC, point-of-care; RPR, rapid plasma reagin; SDI, Sexually Transmitted Diseases Diagnostics Initiative, STI, sexually transmitted infection; TPI, *Treponema pallidum* immobilisation; VCN, vancomycin, colistin and nystatin; VDRL, venereal disease research laboratory; WHO, World Health Organization

As the primary author, I will share insights that have made me value STI diagnostics. During my Infectious Disease and Microbiology fellowships at the University of Washington in the 1960s, I had the wonderful experience of spending 18 months in John Sherris's Microbiology Laboratory at University Hospital. Dr Sherris enabled microbes to be ''virtual participants'' in daily rounds. On my return to Winnipeg with significant laboratory responsibilities, clinical microbiology became personally very rewarding for 15 years. However, when I assumed the Chair in Internal Medicine at Manitoba, clinical microbiology ceased to be part of my daily routine and I missed much of the molecular diagnostics revolution.

In 1980, I was invited to be part of an STI programme with the University of Nairobi. STIs, even without the calamity of HIV, are devastating illnesses in most African societies. Laboratory support is minimal with little or no public health presence in the field of STI diagnostics. Syndromic management of symptomatic STIs is the only management option available. No epidemiologic investigation of STIs occurs outside of research projects. Without laboratory support, STI care becomes a guessing game with many errors in management, including substantial under- and over-treatment. In addition, diagnostic clinical errors are not recognised, even in retrospect. The treatment target becomes large and imprecise, and practitioners do not learn when they have missed it. Furthermore, when we are unaware of what we do not know, the practice of medicine becomes haphazard and chaotic.

This is the experience in much of the resource-limited world with regards to STIs. WHO is correct in the assumption that ''placing increased value on STI diagnosis'' is an essential strategy if we are to successfully reduce the burden of STIs. Perhaps applauding some of the important contributions of the last 50 years will assist in this overall objective.

METHODS

Twenty individuals were invited to independently identify the most important STI diagnostic manuscripts from the past 50 years (1950–2000). They were asked to exclude articles in which they were included as authors. Advances in HIV diagnosis were also excluded. Five individuals submitted nominations (the authors of this manuscript) and two of these individuals selected articles limited to the diagnosis of syphilis. This initial list of 38 articles was distributed to the entire SDI Editorial Board, comprised of STI diagnostics and clinical experts from 17 countries. Board members were asked to rate their top 10 choices in order of perceived importance. Of the 63 experts, 25 voted, for a response rate of approximately 40%. Several board members also nominated additional articles, bringing the total number of articles to 43. We ultimately chose to recognise 13 articles as exceptional. Three manuscript couplets with overlapping authors were chosen in addition to seven ''stand alone'' articles. It could be argued that the couplet pairs are a continuum with both articles interdependent and supporting each other. My own choices (Allan Ronald) included seven of these but I also identified three other favourites and these are identified with my reasons, along with the list of the other 26 nominated in this process (see Appendix).

RESULTS

The results are tabulated in two ways, by total number of votes and by rank score (table 1). The former represents a count score of the total number of votes a particular article received. The rank score was calculated based on the article's perceived importance, so articles ranked 1 received 10 points, 2 received 9, 10 received 1, etc. There was remarkable consistency between these two measures, with eight of the top 10 articles appearing in both lists.

Table 1 Top 10 STI diagnostics publications 1950–2000

Ranking	Top 10 by number of votes	Top 10 by rank score
1	Thayer and Martin 1964	Thayer and Martin 1964
2	Portnoy *et al* 1957	Portnoy *et al* 1957
3	Amsel *et al* 1983	Hunter *et al* 1964
4	Tam *et al* 1984	Tam *et al* 1984
5	Hunter *et al* 1964	Wang *et al* 1970
6	Wang *et al* 1970	Amsel *et al* 1983
7	Ashley *et al* 1985	T'ang 1957
8	Lee *et al* 1985	Lee *et al* 1985
9	Nonnenmacher *et al* 1996	Nelson 1949
10	Bauer *et al* 1991	Ashley *et al* 1985

THE TOP CHOICES BY TOTAL NUMBER OF VOTES

1. Thayer JD, Martin JE Jr. A selected media for the cultivation of *N. gonorrhoeae* and *N. meningitidis*. *Public Health Rep* 1964;49:49–57.

The culture and isolation of *Neisseria gonorrhoeae* is relatively straightforward in men with gonococcal urethritis, but more problematic in cultures obtained from the female genital tract. In 1964 Thayer and Martin, working in the Neisseria Group, Venereal Disease Research Laboratory, National Communicable Disease Center, Atlanta, Georgia described the initial use of antimicrobial agents in media to inhibit the growth of normal flora and facilitate the isolation of gonococci. Initially they chose polymyxin and ristocetin but ultimately determined that vancomycin, colistin and nystatin (VCN) were the antibiotics of choice for the selective cultivation of *N gonorrhoeae*. In a later article, Martin *et al*[4] carried out a field trial with a commercial (Baltimore Biological Laboratory) Thayer/Martin media for the primary isolation of *N gonorrhoeae*. They demonstrated that VCN reduced ''contaminants'' by 76% and increased the number of gonococcal colonies by 172% in cultures obtained from the female genital tract. This resulted in a 78% increase in the number of vaginal/cervical cultures positive for *N gonorrhoeae*. Although subsequent studies demonstrated that a small subset of gonococci were susceptible to vancomycin, overall this medium or its modifications have globally altered cultural strategies for *N gonorrhoeae* and other fastidious organisms, permitting their isolation from contaminated sources. It also improved epidemiologic studies by enabling improved organism recovery on culture. Although nucleic acid technologies are now widely used and are sensitive and specific, cultural techniques remain inexpensive, widely available, effective, and essential if antimicrobial testing is to be undertaken.

2. Portnoy J, Garson W, Smith CA. Rapid plasma reagin test for syphilis. *Public Health Rep* 1957;72:761–6.

Wasserman had initially described a complement fixation test for syphilis using the reagin antigen in 1906.[5] A simplification of this test, the venereal disease research laboratory (VDRL) serologic test for the reagin antigen was used for several decades as the routine screening to diagnose syphilis. The test required freshly made antigen and heat inactivation of the serum sample. In this article Portnoy *et al* described the use of plasma rather than serum and used a cardiolipin antigen suspended in 10% choline chloride solution with added merthiolate. The antigen suspension was stable for some weeks and did not require inactivation of complement. The plasma and antigen suspension was mixed on a mechanical rotator for four minutes and then read microscopically using 100× magnification. The test had improved sensitivity and specificity

compared to earlier tests and a titred test to quantify the antibody was soon developed. Subsequent improvement of the addition of charcoal particles led to a test that could be read macroscopically. With appropriate controls, the rapid plasma reagin (RPR) had the characteristics of a POC test that could be available to provide a diagnosis and treatment simply and at low cost. It revolutionised syphilis screening technology.

3. Amsel R, Totten PA, Spiegel CA, *et al*. Non-specific vaginitis: diagnostic criteria and microbial and epidemiologic associations. *Am J Med* 1983;74:14–22.

Non-specific vaginitis is a common problem for women and their caregivers. Previous studies had identified the transmissibility of pathogens, the predominance of *Gardnerella vaginalis*, and the physical and biochemical characteristics of vaginitis. In their article, Amsel and the Seattle group survey the literature to develop diagnostic criteria. These criteria were further refined in a subsequent article by Spiegel[6] and by Nugent.[7] However, the initial article provided the foundation for a scorecard that Nugent and colleagues standardised and popularised with their article. This continues to be our primary strategy for diagnosing what is now referred to as bacterial vaginosis. Only with a well developed case definition was it possible to proceed with delineation of prevalence, incidence, and possible risk behaviours that are responsible for this incredibly common and still enigmatic condition.

4. Tam MR, Stamm WE, Handsfield HH, *et al*. Culture independent diagnosis of *Chlamydia trachomatis* using monoclonal antibodies. *N Engl J Med* 1984;310:1146–50.

This article simplified the diagnosis of chlamydial infection and enabled rapid diagnosis within hours using fluorescence-labelled monoclonal antibodies that detected extracellular elementary bodies in specimens with a reasonable sensitivity as compared to culture and enabled diagnosis to become more widely available. These fluorescent monoclonal antibodies were also used for culture confirmation, an important step in improving the sensitivity and specificity of culture confirmation compared to iodine staining. Enzyme immunoassays (EIA) replaced this technology later in the decade and were in turn largely supplanted by nucleic acid hybridisation (NAH) and amplification (NAA) technologies in the 1990s. Multiple groups, including commercial enterprises, have enabled a variety of methods to be available for routine laboratory use. NAA technologies have become the gold standard with excellent sensitivity and specificity.

5. Hunter EF, Deacon WE, Meyer PE. An improved FTA test for syphilis: The absorption procedure (FTA-ABS). *Public Health Rep* 1964;79:410–12.

Complex technologies were initially used to identify treponemal antibody in patients with positive syphilis screening tests. The *Treponema pallidum* immobilisation (TPI) test was the standard test but it was only available at a few sites and required live treponemes and a great deal of technological infrastructure.[8] The fluorescent treponemal antibody (FTA) test had been described by investigators at the Communicable Disease Center but it was too non-specific despite several modifications.[8] In this paper, Hunter *et al* described a modification that absorbed the serum with a sonicate of Reiter treponeme (*T phagedenis*) which removed non-specific antibody and led to a specific test which was shown to be sensitive for treponemal antibody. Of interest in this paper, sera from the Tuskegee study of the natural history of syphilis were used to validate the results. Overall, this modification of the FTA test for specific treponemal antibody remained the standard until the 1980s when more rapid tests were identified.[9]

6. Wang SP, Grayston JT. Immunologic relationship between genital TRIC, lymphogranuloma venereum and related organisms in a new microtitre indirect immunofluoresence test. *Am J Ophthalmol* 1970;70:367–75.

Wang SP, Grayston JT. Human serology in *Chlamydia trachomatis* infection with microimmunofluorescence test. *J Infect Dis* 1974;130:388–97.

Schachter and others identified *Chlamydia trachomatis* as a major cause of STIs during the 1970s.[10] Cultural diagnosis was challenging and insensitive largely due to stringent transport requirements. The microimmunofluorescence (MIF) technique developed by Wang and Grayston was the basis for many of the early epidemiologic studies that contributed to our understanding of the relationships not only between chlamydia species but also between ocular and urogenital strains of *C trachomatis*. This is also the technique that enabled later development of the fluorescence assay, based on monoclonal antibodies, for earlier recognition of *C trachomatis* in cell culture and dramatically increased sensitivity over earlier iodine and Giemsa staining techniques. In 1989, MIF was one of the techniques used to define *C pneumoniae* as a new species of chlamydiae.

7. Ashley RL, Militoni J, Lee FK, *et al*. Comparison of Western blot (immunoblot) and glycoprotein G-specific immunodot enzyme assay for detecting antibodies to herpes simplex virus types 1 and 2 in human sera. *J Clin Microbiol* 1988;26:662–7.

Lee FK, Coleman M, Pereira L, *et al*. Detection of herpes simplex virus type 2 - specific antibody with glycoprotein G. *J Clin Microbiol* 1985;22:641–4.

During the 1970s it was recognised that the herpes simplex virus (HSV) could be typed into two species which differed epidemiologically. However, HSV-2, the predominant cause of genital herpes, could not be readily differentiated on the basis of serologic response. Ashley *et al* demonstrated that by using neutralisation assays and Western blot, unique antibodies could be identified to distinguish HSV-1 from HSV-2. The identification of antibody to HSV-2 glycoprotein G and the subsequent study correlating HSV glycoprotein antibody with a specific Western Blot response initiated an era in which the glycoprotein has become the primary technical strategy to diagnose HSV-2 infection. Several commercial technologies are currently available to identify antibody to glycoproteins G1 and G2. This has led to epidemiologic studies that have substantiated the relationship of HSV-2 to HIV transmission and have also permitted large scale epidemiologic prevalence studies to be carried.

8. Kirnbauer R, Hubbert NL, Wheeler CM, *et al*. A virus-like particle enzyme-linked immunosorbent assay detects serum antibodies in a majority of women infected with human papillomavirus type-16. *J Natl Cancer Inst* 1994;86:494–8.

Nonnenmacher B, Kruger Kjaer S, Svare EI, *et al*. Seroreactivity to HPV16 virus-like particles as a marker for cervical cancer risk in high-risk populations. *Int J Cancer* 1996;68:704–9.

Although a viral aetiology for warts had been suggested in 1907, and the sexual route was proven in 1954, the failure to cultivate human papillomavirus (HPV) limited the development of technologies to diagnose HPV infection. Nucleic acid technologies, including in situ hybridisation, enabled epidemiologic studies and substantiated the association with neoplasia. However, this was largely a research technology, as noted in the subsequent article by Bauer *et al*. Kirnbauer *et al*

expressed recombinant HPV-16 capsid proteins in a Baculovirus expression system and demonstrated that type restricted HPV serology could be determined. Anticapsid seropositivity correlated with type specific HPV infection. This technology was rapidly exploited over the next decade to determine the epidemiology of HPV-16, HPV-18, and other oncogenic HPV isolates. From our perspective, the key serologic piece to this story was elicited by Kirnbauer and his colleagues in the work they pursued to clone and manufacture quantities of the capsid proteins for antigenic use of the serologic test.

In our second HPV manuscript, Nonnenmacher *et al* developed an enzyme-linked immunosorbent assay (ELISA) based on HPV-16 viral particles and explained the previous lack of correlation between HPV DNA prevalence and cervical cancer risk in high-risk populations. In earlier reports, seropositivity was strongly associated with detection of HPV-16 DNA in low-risk populations. In the present study conducted on a high-risk sexually transmitted disease clinic population of women in Greenland and Denmark, antibody to HPV-16 was found above a previously defined cut-off in 56% of Greenland women and 41% of Danish women. Whereas the detection of HPV-16 DNA from the female genital tract decreased with age, seroreactivity to HPV-16 increased with age and in the Danish women correlated with the number of life-time sex partners. Progress has occurred rapidly since and within the next 1–2 years a vaccine for HPV will be available which will almost certainly prevent most cervical cancer. This was only possible with the initial recognition of HPV as an STI in the female genital tract, its oncogenic relationship to cervical cancer and other epithelial squamous cell cancers, and ultimately the ability to understand its epidemiology.

9. Bauer HM, Ting Y, Greet CE, *et al*. Genital human papillomavirus infection in female university students as determined by a PCR-based method. *JAMA* 1991;265:471–6.

HPV DNA was initially identified in genital specimens through immunocytochemistry and DNA hybridisation. In this study, Bauer *et al* describe a polymerase chain reaction (PCR)-based method to identify the HPV region which encodes the viral capsid protein. Specimens were taken from the female genital tract and analysed with PCR amplification. Among 467 female college students, 46% were infected with HPV. Although previous studies, particularly those carried out by the University of Washington group, had identified the epidemiology of HPV and its association with cervical neoplasm, the study simplified the technology and demonstrated that their PCR technique had increased sensitivity, correlated with the sexual history, and described and identified HPV types.[11]

10. Scholes D, Stergachis A, Heidrich FE, *et al*. Prevention of pelvic inflammatory disease by screening for cervical chlamydial infection. *N Engl J Med* 1996;334:1362–6.

Ultimately diagnostic technologies are only useful if they contribute to disease control and prevention, as well as contributing to a clinical diagnosis for the individual patient. In this study the Seattle group identified 2607 eligible women who were randomised to *C trachomatis* screening or ''usual care''. The patients who were screened and found to be positive for *C trachomatis* were offered treatment. A strategy of identifying women at risk, testing for chlamydia, and treating those who were infected, reduced by over 50% the subsequent occurrence of pelvic inflammatory disease in this population of at-risk women. This important study emphasises the need to screen for *C trachomatis* infection in women with identified risk behaviours to reduce the unnecessary complication of pelvic inflammatory disease and its potential complications. These and numerous other studies have provided evidence that verifies public health initiatives to screen and treat individuals, which can be widely exploited for disease control. Multiple studies carried out by other authors could also be cited. However, the Seattle group has been effective in utilising these technologies regionally and demonstrating that this approach could reduce the prevalence of *C trachomatis* as well as the complications of this insidious infection.

THREE ALTERNATE CHOICES BY ALLAN RONALD AND THE ARGUMENT FOR THEIR ''INCLUSION'' AS IMPORTANT MILESTONES IN STI DIAGNOSIS

Ostergaard L, Mooler JK, Andersen B, *et al*. Diagnosis of urogenital *Chlamydia trachomatis* infection in women based on mailed samples obtained at home: multi-practice comparative study. *BMJ* 1996;313:1186–9.

The introduction of nucleic acid technologies for *C trachomatis* and *N gonorrhoeae* have enabled a shift to occur in strategies for obtaining specimens. During the 1990s it was recognised that urine was a readily accessible, more user friendly source for detection of these pathogens, but sensitivity was reduced. However, the strategy of self-obtained vaginal specimens and mailing them to the laboratory made it possible for women to be diagnosed specifically with minimal interference and very little additional cost for the laboratory investigation. No longer are an appointment, a journey, usually a prolonged wait, and the cost of the examination inherent to obtaining an adequate specimen. In this study, Ostergaard *et al* carried out a test for *C trachomatis* in 222 women in Aarhus County, Denmark. They compared the diagnostic efficacy of samples obtained by women at home with a vaginal irrigation technology and mailed to the laboratory to those obtained by a doctor using a cervical swab in the office. The results of the two collection methods were equivalent. Simple collection methods with widespread introduction and with the support of women's groups and those at risk of STIs could become the foundation for regional, national, and ultimately global control of *C trachomatis* and perhaps *N gonorrhoeae*.

Romanowski B, Sutherland R, Fick GH, *et al*. Serologic response to treatment of infectious syphilis. *Ann Intern Med* 1991;114:1005–9.

Romanowski and colleagues reviewed the serologic outcomes of 1090 patients with primary, secondary, and early latent syphilis seen in Alberta, Canada. In follow-up they noted that initial primary and secondary infections were more likely to serorevert and that the RPR test seroreversal rates depended upon the pre-treatment titre, with higher titres less likely to serorevert. RPR seroreversion was unusual with early latent disease. In this study, 72% of patients with primary syphilis and 56% of patients with secondary syphilis reverted to non-reactive by 36 months. Of interest, the treponemal antibody tests (fluorescent treponemal antibody absorption test (FTA-ABS) and microhaemagglutination assay for *T pallidum* antibodies (MHA-TP)) also seroreverted (24% non-reactive at 36 months for the FTA-ABS and 13% for the MHA-TP). Only patients with initial episodes of syphilis became seronegative. Antibody titres decline more slowly in early latent syphilis and all treponemal antibody tests remained positive at 36 months for patients with a diagnosis of latent syphilis.

Orle, KA, Gates CA, Martin DH, *et al*. Simultaneous PCR detection of *Haemophilus ducreyi*, *Treponema pallidum*, and herpes simplex virus types 1 and 2 from genital ulcers. *J Clin Microbiol* 1996;34:49–54.

Multiplex PCR (M-PCR) is the simultaneous co-amplification and identification of different DNA targets in the same

amplification tube. The amplifications proceed independently of each other, but the primers for each of the pathogens need to share characteristics such as size and melting temperatures, and the assay requires a detection step that can identify each amplicon. This technique was initially used to detect *C trachomatis* and *N gonorrhoeae* simultaneously in genital specimens or urine as single or co-infections. Roche Diagnostics developed an M-PCR assay for genital ulcer disease with primers that would identify four pathogens; *T pallidum*, *H ducreyi*, and HSV 1 and 2. In this study Orle *et al* demonstrated the sensitivity and specificity of this technique and its potential usefulness as a rapid, reasonably accurate method to diagnose the pathogen(s) present in genital ulcers. Although widely used as a research tool, it has not been marketed presumably because of the relatively small commercial market for the diagnosis of genital ulcer disease in the developed world.

DISCUSSION

This effort highlights some of the important original contributions to the STI diagnostic literature over the past half century. Of the 38 nominations for these ''Oscars'', only one was voted for unanimously by all 25 participants as a classic. We each have our own favourites and the choices were more eclectic than we anticipated. We assume many of our readers would have made other selections. However, this process has identified significant advances that identified new technologies and pathogens and radically changed the STI diagnostics world. Laboratory investigation is essential for optimal STI patient care and control strategies. Most of these have become more or less routine in the developed world, both for the investigation of individual patients and for public health interventions.

Substantially more translational research needs to be done to fully develop STI diagnostics and further transform care and prevention by enabling screening to take place with home kits and unobstructed access to diagnostics. With nucleic acid technologies, sensitivity and specificity are usually not an issue in laboratories with excellent quality assurance and quality control. However, robust platforms and easily interpreted end points require further development.

Presumably more sexually transmitted pathogens will be identified and we will identify new pathogens for several disease syndromes where aetiologic gaps still exist. Only through careful prospective studies of clinical syndromes with well planned comprehensive laboratory investigation will we identify what is still unknown.

The need for major breakthroughs in new technology is most urgent in the resource constrained world. Simple, sensitive, specific, inexpensive, POC technologies with robust platforms need to be designed and validated by an international regulatory process that is supportive and collegial but sufficiently authoritative to have widespread credibility. The skills required to perform and interpret the tests must be easily learned and require limited supervision and minimal maintenance of laboratory equipment. In 2006, these goals should be feasible with the rapid advances occurring in biotechnology. The STD Diagnostics Initiative in WHO/TDR will aid this process. The costs of discovery and development need to be met by both research agencies and international donors. Presumably there needs to be opportunity for the private sector within countries to have an appropriate role in marketing and delivery of diagnostic products to enable reliable distribution to the end user. Understanding the obstacles to the use of diagnostics in developing countries and a means to overcome these barriers are urgently needed.

Perhaps five years from now, innovative technologies for STIs and their use to dramatically reduce the prevalence of STIs in developing countries will be heralded as the significant diagnostic advances of this decade.

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REFERENCES

1 **Ndinya-Achola JO**, Kihara AN, Fisher LD, *et al*. Presumptive specific clinical diagnosis of genital ulcer disease (GUD) in a primary health care setting in Nairobi. *Int J STD AIDS* 1996;**7**:201–5.
2 **Dallabetta GA**, Gerbase AC, Holmes KK. Problems, solutions, and challenges in syndromic management of sexually transmitted diseases. *Sex Tranms Infect* 1998;**74**:S1.
3 **Markel H**. Who's on first? – medical discoveries and scientific priority. *N Engl J Med* 2004;**351**:2792–4.
4 **Martin JE**, Billings TE, Hackney JF, *et al*. Primary isolation of *N. gonorrhoeae* with a new commercial medium. *Public Health Rep* 1967;**82**:361–3.
5 **Wassermann A**. *Eine serodiagnostische Reaktion bei Syphilis*. Leipzig: George Thieme, 1906.
6 **Spiegel CA**, Amsel R, Holmes KK. Diagnosis of bacterial vaginosis by direct gram stain of vaginal fluid. *J Clin Microbiol* 1983;**18**:170–7.
7 **Nugent RP**, Krohn MA, Hillier SL. Reliability of diagnosing bacterial vaginosis is improved by a standardized method of gram stain interpretation. *J Clin Microbiol* 1991;**29**:297–301.
8 **Nelson RA**, MM Mayer. Immobilization of *Treponema pallidum* by antibody produced in syphilitic infection. *J Exp Med* 1949;**89**:369–93.
9 **Castro RR**, Prieto ES, Santo I, *et al*. Evaluation of the passive particle agglutination test in the serodiagnosis and follow-up of syphilis. *Am J Clin Pathol* 2001;**116**:581–5.
10 **Schachter J**. Chlamydia infections. *N Engl J Med* 1978;**298**:428–35.
11 **Carter JJ**, Koutsky LA, Wipf GC, *et al*. The natural history of human papillomavirus type 16 capsid antibodies among a cohort of university women. *J Infect Dis* 1996;**174**:927–36.

APPENDIX

ADDITIONAL ARTICLES NOMINATED FOR THE ''TOP 10 ADVANCES IN STI DIAGNOSTICS''

- Barre-Sinoussi F, Chermann JC, Rey F, *et al*. Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). *Science* 1983;**220**:868–71.
- Borchardt KA, Smith RF, An evaluation of an InPouch TV culture method for diagnosing *Trichomonas vaginalis* infection. *Genitourin Med* 1991;**67**:149–52.
- Carter JJ, Hagensee MB, Lee SK, *et al*. Use of HPV 1 capsids produced by recombinant vaccinia viruses in an ELISA to detect serum antibodies in people with foot warts. *Virology* 1994;**2**:284–91.
- Carter JJ, Koutsky LA, Wipf GC, *et al*. The natural history of human papillomavirus type 16 capsid antibodies among a cohort of university women. *J Infect Dis* 1996;**174**:927–36.
- Chernesky MA, Jang D, Lee H, *et al*. Diagnosis of *Chlamydia trachomatis* infection in men and women by testing first-void urine by ligase chain reaction. *J Clin Microbiol* 1994;**32**:2682–5.
- Cox P, Logan MLC, Norins LC. Automated, quantitative microhemagglutination assay for *Treponema pallidum* antibodies. *Appl Microbiol* 1969;**18**:485–9.
- Dille BJ, Butzen CC, Birkenmeyer LG. Amplification of *Chlamydia trachomatis* DNA by ligase chain reaction. *J Clin Microbiol* 1993;**31**:729–31.

- Falcone VH, Stout GW, Morre MBM. Evaluation of rapid plasma reagin (circle) card test. *Public Health Rep* 1964;**79**:491–5.
- Gallo RC, Salahuddin SZ, Popovic M, *et al*. Frequent detection and isolation of cytopathic retroviruses (HTLV-III) from patients with AIDS and at risk for AIDS. *Science* 1984 4;**224**:500–3.
- Gift TL, Pate MS, Hook EW 3rd, *et al*. The rapid test paradox: when fewer cases detected lead to more cases treated: a decision analysis of tests for *Chlamydia trachomatis*. *Sex Transm Dis* 1999;**26**:232–40.
- Hammond GW, Lian CJ, Wilt, *et al*. Comparison of specimen collection and laboratory techniques for isolation of *Haemophilus ducreyi*. *J Clin Microbiol* 1978;**7**:39–43.
- Hanff, PA, Fehniger TE, *et al*. Humoral immune response in human syphilis to polypeptides of *Treponema pallidum*. *J Immunol* 1982;**129**:1287.
- Lorincz A. Detection of human papillomavirus DNA without amplification: prospects for clinical utility. *IARC Scientific Publications* 1992;**119**:135–45.
- Marra CM, Maxwell CK, Smith SL, *et al*. Cerebrospinal fluid abnormalities in patients with syphilis: association with clinical and laboratory features. *J Infect Dis* 2004;**189**:369–76.
- Mayaud P, Grosskurth H, Changalucha J, *et al*. Risk assessment and other screening options for gonorrhoea and chlamydial infections in women attending rural Tanzanian antenatal clinics. *Bull World Health Organ* 1995;**73**:621–30.
- Nelson RA, MM Mayer. Immobilization of *Treponema pallidum* by antibody produced in syphilitic infection. *J Exp Med* 1949;**89**:369–93.
- Norgard MV, Selland CK, Kettman JR, *et al*. Sensitivity and specificity of monoclonal antibodies directed against antigenic determinants of *Treponema pallidum Nichols* in the diagnosis of syphilis. *J Clin Microbiol* 1984;**20**:711–17.
- Nugent RP, Krohn MA, Hillier SL. Reliability of diagnosing bacterial vaginosis is improved by a standardized method of gram stain interpretation. *J Clin Microbiol* 1991;**29**:297–301.
- Percival A, Corkill JE, Aryao P, *et al*. Penicillinase-producing gonococci in Liverpool. *Lancet* 1976;ii:1379–80.
- Ripa KT, Mardh PA, Cultivation of *Chlamydia trachomatis* in cycloheximide-treated McCoy cells. *J Clin Microbiol* 1977;**6**:328–31.
- Roizman B, Keller JM, Spear PG, *et al*. Variability, structural glycoproteins, and classification of herpes simplex viruses. *Nature* 1970;**227**:1253–4.
- Sarngadharan MG, Popovic M, Bruch L, *et al*. Antibodies reactive with human T-lymphotropic retroviruses (HTLV-III) in the serum of patients with AIDS. *Science* 1984;**224**: 506–8.
- Skinner GR, Hartley CH, Whitney JE. Detection of type-specific antibody to herpes simplex virus type 1 and 2 in human sera by complement-fixation tests. *Arch Virol* 1976;**50**:323–33.
- Spiegel CA, Amsel R, Holmes KK. Diagnosis of bacterial vaginosis by direct gram stain of vaginal fluid. *J Clin Microbiol* 1983;**18**:170–7.
- T'ang FF, *et al*. Trachoma virus in chick embryo. *Natl Med J China* 1957;**43**:81.
- Terry G, Ho L, Londesborough P, *et al*. Detection of high-risk HPV types by the hybrid capture 2 test. *J Med Virol* 2001;**65**:1555–62.
- Tully JG, Taylor-Robinson D, Cole RM, *et al*. A newly discovered mycoplasma in the human urogenital tract. *Lancet* 1981;i:1288–91.
- Veldkamp J, Visser AM. Application of the enzyme-linked immunosorbent assay (ELISA) in the serodiagnosis of syphilis. *Br J Venereal Dis* 1975;**51**:227–31.